DIFFERENTIAL NUCLEIC ACID METABOLISM OF PLANARIAL SEGMENTS

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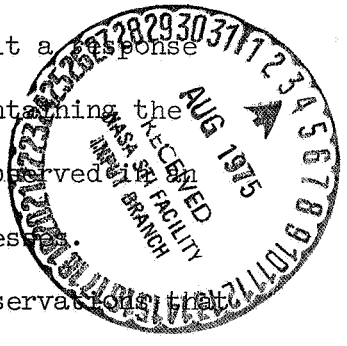
Abstract. Incorporation of cytidine-C^{14} into the RNA of the "head" segment of planaria is inhibited by tricyanoaminopropene whereas other nucleic acid bases are unaffected. This may be related to the content in this segment of neurons with short processes.

Recent studies (1) by Best and Elshtain have shown that in planaria, electrical discharge of different classes of neurons in the animal can be related to the voltage used to produce an unconditioned response. This was attributed to a relatively low rheobasic intensity and long chronaxie in the long neurons compared with short neurons. It was demonstrated (1,2) that neurons in the head of planaria have processes about 4 μ long whereas the processes are at least 30 μ long in the trunk. These results suggest that other phenomena in the planaria may be related to these differences and that compounds known to selectively affect nervous tissue metabolism might influence the two classes of neurons differently.

We have been investigating differential biochemical activities in various segments of the planaria and would like to present data here which demonstrate that the compound 1,1,3-tricyano-2-aminopropene (TCAP) can elicit a response in the nucleic acid metabolism of that portion of the animal containing the major proportion of neurons with short processes which is not observed in the immediately posterior portion containing neurons with long processes.



The choice of TCAP as the test compound was dictated by observations that it has a profound action on the nucleic acid metabolism of nerve cells. It is

capable of producing a rapid increase in the content of nucleic acids in certain types of rabbit nerve cells in vivo (3). This increase is associated with changes in the base composition of the RNA (4). It also accelerates the growth of nerve processes in the newt and the outgrowth of fibers from chick ganglia in vitro (5). These observations may be related in some way to the report (6) that TCAP produces, in rats, an enhanced retention of learning in an avoidance conditioning situation and the observation (7) that it also decreases retrograde amnesia produced by electroconvulsive shock of a newly learned passive avoidance response. Here, animals (8), partially immobilized by cooling to 2°C for 20 minutes, were cut under a low power dissecting microscope immediately posterior to the auricles to give a "head" section and then a subsequent "neck" section of as nearly equal volume as possible. Within a given experiment, care was taken to select specimens of the same size.

For each experimental condition, nine portions of animal were randomly selected and placed, in groups of three, into three 15 × 100 mm tubes containing 0.2 ml of medium containing radioactive tracer and other addenda. The tracers used had the following specific activities: H^3-uridine (15.3 mc/mg), H^3-cytidine (25.0 mc/mg), adenine-2-C^{14} (0.059 mc/mg) and guanine-2-C^{14} (0.022 mc/mg). Each tube contained either a combination of 1.0 μc each of H^3-uridine and C^{14}-adenine or of H^3-cytidine and C^{14}-guanine. TCAP (9) was dissolved in water to give a 0.25 percent solution and stored in the dark at -20°C.

The medium used is capable of maintaining disrupted planarial tissue in culture for up to two weeks. It was developed from the salt solution described by Henderson and Eakin (10) and had the following composition: $CaCl_2$ (10^{-2}M), KCl (10^{-5}M), NaCl (10^{-3}M), disodium versenate (10^{-5}M), Eagle's MEM amino acids, fetal bovine serum (10 percent), penicillin

(100 U/ml), streptomycin (0.1 mg/ml), glucose (5 mg/ml) with the final pH adjusted to 7.4 with 7.5 percent $NaHCO_3$. TCAP was added to the experimental tubes to give final concentrations of 25 μg/ml and 100 μg/ml.

The capped tubes were maintained in the dark at 17° for 18 hours and the incubations were then terminated by washing twice with cold phosphate-buffered saline. All segments of the animals were still mobile at this time. The tissues were then treated according to the procedure of Scott, et al. (11) to give low molecular weight (LMW), RNA, and combined DNA-protein fractions. The optical density of the LMW and RNA fractions was determined in a Zeiss PMQ II spectrophotometer using microcuvettes. The radioactivity of an aliquot of these fractions was determined by liquid scintillation counting. The combined DNA-protein precipitate was dissolved in 0.1 ml of 90 percent formic acid and also counted.

Data from four different experiments conducted under identical conditions have been combined. Unfortunately, it was not possible to obtain identically sized animals for all four experiments and much of the statistical variation is probably due to this. The optical densities of the LMW and RNA fractions are shown in Table 1. It can be concluded that the "neck" segments were larger than the "head" segments, which is to be expected since the animal becomes thicker toward the middle. The effect of TCAP was minimal, and if anything, caused a decrease to the same extent in both segments of UV absorbing material. The ratio of absorbing material in the two fractions was different in the "head" and "neck" segments which is probably a reflection of the different cell types present with differing pool sizes and metabolic activities.

Table 1

The incorporation of the radioactive precursors into the RNA fraction is shown in Table 2. In the controls, about 0.5 percent of the radioactivity present in the medium was incorporated. Only in the "head" segment was there

Table 2

any effect of TCAP on the incorporation of the radioactive precursors. The incorporation of cytidine was inhibited 40 percent by 100 μg/ml of TCAP. The incorporation of all of the precursors was significantly greater into the "head" portions and again indicates a different cell population in the two segments.

The inhibition of cytidine incorporation into the RNA of the "head" segments could have arisen from its unavailability in the LMW fraction. Such was indeed the case since 100 μg/ml of TCAP caused a 57 percent decrease in the amount of radioactivity in the LMW fraction when radioactive cytidine was present in the medium. No inhibition of any of the other precursors was found in either the "head" or "neck" portions. There did appear to be about a 20 percent stimulation of uridine incorporation into the "head" section produced by 100 μg/ml of TCAP which was not found in the "neck" section. This increase in uridine incorporation would be expected in the presence of a specific inhibition of cytidine phosphorylation since an increased amount of UMP would be converted to CMP in this situation.

The content of radioactivity in the combined DNA-protein fraction was quite low. The only change produced by 100 μg/ml of TCAP was a greater than 90 percent inhibition of the incorporation of cytidine into this fraction of the "head" segments which was not observed in the "neck" segments.

The mechanism by which TCAP affects nucleic acid metabolism is not known. Perhaps the most revealing observation (12) is that TCAP can reverse the inhibition by actinomycin D of the incorporation of uridine into dipteran salivary gland nucleolar and chromosomal RNA. Since in the absence of actinomycin D there was also a stimulation of uridine incorporation, there is probably a direct effect of TCAP on DNA-dependent RNA synthesis. It has also been reported (13) that TCAP at ten times the concentration of 2,4-dinitrophenol

can uncouple oxidative phosphorylation in rat liver mitochondria. However, these studies were not conducted with nervous tissue where it is probable that rather specific effects occur, since only certain types of rabbit nerve cells showed an increase in UV absorbing material when TCAP was administered (3).

It may be particularly significant that in one of the affected cell types, the neurons in the Deiter's nucleus, that TCAP caused a specific decrease in the amount of cytosine relative to the other bases in spite of a net increase of 26 percent in the net content of RNA in these cells (4). It was also reported that although the UV absorption was increased for the whole cell, the increase was particularly great in the nucleus.

The results reported here are also consistent with data obtained with frog ganglion tissue maintained in vitro where it was shown (14) that comparable concentrations of TCAP inhibited the incorporation of cytidine and to a lesser extent adenine into RNA. In this tissue there was also an inhibition of the incorporation of cytidine into the LMW fraction.

It is of course possible that the effect of TCAP observed here is not due to action on the different types of nerve cells of the two segments, but rather to some other difference. The possibility that regenerating cells (neoblasts) are incorporating the radioactivity is contraindicated by the evidence (15) that there is only about a 10 percent greater content in the "head" than in the "neck" whereas the difference observed here in incorporation into RNA is at least 200 percent greater. Further, the "neck" has two cut and presumably regenerating surfaces and might thus be expected to have a higher rate of regeneration of neoblasts. Finally, the incorporation of the precursors into the DNA-protein fraction of the segments was essentially the same. That there is indeed a gradient in metabolic activity in planaria has been established (16) but insofar as it involves the incorporation of

radioactive CO_2 and glycine into protein can be attributed to the activity of a variety of cell types, and certainly does not explain why the incorporation of cytidine should be selectively inhibited in the "head" segments by TCAP.

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Table 1. Optical density of planarial segment fractions.

Fraction	Optical density	
	Head	Neck
LMW-control	0.077 ± 0.008	0.124 ± 0.012
LMW-25 μg/ml TCAP	0.078 ± 0.007	0.128 ± 0.011
LMW-100 μg/ml TCAP	0.062 ± 0.009	0.115 ± 0.008
RNA-control	0.119 ± 0.009	0.248 ± 0.019
RNA-25 μg/ml TCAP	0.123 ± 0.014	0.263 ± 0.023
RNA-100 μg/ml TCAP	0.095 ± 0.016	0.226 ± 0.023

Each value represents the mean ± standard error of the mean for 72 segments as determined in groups of 3.

Table 2. Effect of TCAP on the RNA metabolism of planaria segments.

Addition	Incorporation* (cpm/unit O.D. 260 mμ)			
	Uridine	Adenine	Cytidine	Guanine
	Head			
None	2650 ± 420	2200 ± 250	1500 ± 170	950 ± 85
TCAP, 25 μg/ml	2150 ± 325	1600 ± 800	1350 ± 200	1000 ± 80
TCAP, 100 μg/ml	2700 ± 530	2650 ± 420	900 ± 265	790 ± 105
	Neck			
None	565 ± 100	725 ± 100	505 ± 120	485 ± 40
TCAP, 25 μg/ml	460 ± 55	745 ± 40	550 ± 115	515 ± 40
TCAP, 100 μg/ml	620 ± 90	620 ± 45	550 ± 45	600 ± 45

*The numbers represent the mean incorporation of each of 36 segments treated in groups of 3 ± the standard error of the mean.

Effect of TCAP on the incorporation of nucleic acid bases into the LMW fraction of planaria segments.

Addition	Incorporation* (cpm/unit O.D. 260 mμ)			
	Uridine	Adenine	Cytidine	Guanine
	Head			
None	2550 ± 130	10,000 ± 780	7300 ± 1150	5200 ± 520
TCAP, 25 μg/ml	3100 ± 130	10,400 ± 515	4700 ± 960	3450 ± 450
TCAP, 100 μg/ml	3400 ± 485	11,300 ± 1050	3150 ± 645	5950 ± 1150
	Neck			
None	1950 ± 240	4350 ± 240	4450 ± 1300	2100 ± 320
TCAP, 25 μg/ml	2750 ± 390	5200 ± 590	7700 ± 1900	1950 ± 550
TCAP, 100 μg/ml	1900 ± 175	4850 ± 305	4950 ± 950	2050 ± 435

*The numbers represent the mean incorporation of each of 36 segments treated in groups of 3 ± the standard error of the mean.

Effect of TCAP on the incorporation of nucleic acid precursors into planaria combined DNA and protein fraction.

Addition	Incorporation* (cpm) per segment			
	Uridine	Adenine	Cytidine	Guanine
	Head			
None	28 ± 5	71 ± 12	33 ± 9	125 ± 43
TCAP, 25 μg/ml	27 ± 7	84 ± 29	19 ± 7	103 ± 25
TCAP, 100 μg/ml	23 ± 6	43 ± 9	2 ± 1	65 ± 25
	Neck			
None	23 ± 11	73 ± 20	17 ± 11	65 ± 13
TCAP, 25 μg/ml	19 ± 5	86 ± 21	35 ± 11	80 ± 18
TCAP, 100 μg/ml	28 ± 8	62 ± 15	15 ± 3	62 ± 11

*The numbers represent the mean incorporation of each of 36 segments treated in groups of 3 ± the standard error of the mean.